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Title:

Clinical and molecular description of 19 patients with GATAD2B-Associated Neurodevelopmental Disorder (GAND)

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Abstract

De novo pathogenic variants in the *GATAD2B* gene have been associated with a syndromic neurodevelopmental disorder (GAND) characterized by severe intellectual disability (ID), impaired speech, childhood hypotonia, and dysmorphic features. Since its first description in 2013, nine patients have been reported in case reports and a series of 50 patients was recently published, which is consistent with the relative frequency of *GATAD2B* pathogenic variants in public databases. We report the detailed phenotype of 19 patients from various ethnic backgrounds with confirmed pathogenic *GATAD2B* variants including intragenic deletions. All individuals presented developmental delay with a median age of 2.5 years for independent walking and of 3 years for first spoken words. *GATAD2B* variant carriers showed very little subsequent speech progress, two patients over 30 years of age remaining non-verbal. ID was mostly moderate to severe, with one profound and one mild case, which shows a wider spectrum of disease severity than previously reported. We confirm macrocephaly as a major feature in GAND (53%). Most common dysmorphic features included broad forehead, deeply set eyes, hypertelorism, wide nasal base, and pointed chin. Conversely, prenatal abnormalities, non-cerebral malformations, epilepsy, and autistic behavior were uncommon. Other features included feeding difficulties, behavioral abnormalities, and unspecific abnormalities on brain MRI. Improving our knowledge of the clinical phenotype is essential for correct interpretation of the molecular results and accurate patient management.

Keywords: *GATAD2B*, developmental delay, intellectual disability, next-generation sequencing.

Introduction

The GATA zinc finger domain containing 2B (*GATAD2B*) gene encodes p66beta, a subunit of the transcription repressor complex MeCP1-Mi-2/Nucleosome remodeling and deacetylase (NuRD) (Brackertz et al., 2002; Feng and Zhang, 2001). P66beta has two conserved regions (CR), CR1 interacts with MBD2 and MBD3 proteins, and CR2 interacts with DNA, histone tails (Brackertz et al., 2006) and the CHD4 protein (Torrado et al., 2017). P66beta, like its paralog p66alpha, acts as an enhancer for the NuRD complex repression effect on transcription (Torchy et al., 2015). The role of p66beta in cognition, neuronal plasticity and synapse morphology is supported by studies in the *Drosophila* ortholog Simjang (*simj*) (Willemsen et al., 2013). Ten years ago, a first *de novo GATAD2B* premature termination codon (PTC) variant was reported in a patient with a neurodevelopmental disorder (Vissers et al., 2010). Three additional patients were subsequently reported as carrying either a similar PTC variant or a 1q21.3 microdeletion encompassing *GATAD2B*, allowing the description of a recognizable syndrome (de Ligt et al., 2012; Willemsen et al., 2013) named *GATAD2B*-Associated Neurodevelopmental Disorder (GAND). It is now acknowledged that *GATAD2B* loss of function (LoF) is responsible for the main phenotypic traits of the 1q21.3 microdeletion syndrome (Shieh et al., 2020; Tim-Aroon et al., 2017; Willemsen et al., 2013). Since then, more than 50 LoF variants have been reported in patient-specific databases including DECIPHER (Firth et al., 2009), the Human Genome Mutation Database (HGMD, (Stenson et al., 2017)) and ClinVar (Landrum et al., 2018), suggesting that GAND is not among ultra-rare diseases.

Contrasting with this relatively high frequency in patient-specific databases, the detailed phenotype associated with *GATAD2B* pathogenic variants had been reported in a few patients only. While gathering clinical data for the current study, a series of 50 cases was published, confirming that GAND is not among the rarest causes of neurodevelopmental disorders of

genetic origin (Shieh et al., 2020). The main features of GAND include global developmental delay, impaired speech, severe ID, childhood hypotonia, macrocephaly and dysmorphic features.

We collected the clinical data of 19 patients with the aim to refine the GAND phenotype in order to improve clinical knowledge and management for caregivers and families.

Subjects and Methods

Clinical data of *GATAD2B* pathogenic or likely pathogenic variant carriers were collected from France, Germany, Belgium, United Kingdom, and Italy from May 2018 to November 2019. Referring clinicians were contacted through the AnDDI-Rares platform, a French network for rare diseases, and from other European countries through the GeneMatcher website (Sobreira et al., 2015). Parents or legal representatives previously gave informed written consent for genetic analyses. Additional specific written consent was obtained from parents for the use of patient's photographs. Clinical data was collected through a questionnaire filled by the referring clinician. This study was approved by the Rouen ethics committee. Clinical data were collected from patient's medical charts.

Postnatal growth measurements are reported by Z-score in standard deviation (SD) according to French reference charts or using a calculator based on the WHO Charts for Canada (available at http://www.bcchildrens.ca/health-professionals/clinical-resources/endocrinology-diabetes/tools-calculators#Anthro--calculators). Prenatal measurements were collected, percentiles and Z-scores were calculated using a calculator based on the 2013 Fenton growth charts (available at https://www.ucalgary.ca/fenton/2013chart).

Variants were identified in clinical laboratories following massive parallel sequencing of gene panels or whole exomes or genome sequencing. Gene panel sequencing methods concerning

patient 18 were previously reported (Aspromonte et al., 2020). Interpretation was performed according to the American College of Medical Genetics and Genomics- Association for Molecular Pathology (ACMG-AMP) classification (Richards et al., 2015). Copy number variants were identified using read-depth comparison tools and confirmed by a targeted technique. We did not include genomic deletions encompassing other genes than *GATAD2B*.

Results

General description

We recruited 19 unrelated individuals, 12 males and 7 females, including 13 patients from France (12 Caucasian patients and one of Malagasy origin (patient 19)), three from Germany (patients 12, 16 and 17), one from Belgium (patient 14), one from Italy (patient 18), and one from United Kingdom of Bakhtiari-Iranian ancestry (patient 13). The oldest individual (patient 15) was aged 36 years at the time of diagnosis and 35 years at last examination. The youngest individual (patient 5) was aged 19 months at the time of diagnosis and 2 years and 10 months at last examination.

Molecular genetic analysis

All patients carried premature termination codon (PTC) variants predicted to result in nonsense mediated decay and hence LoF, with 10 (53%) nonsense single nucleotide variants (SNV), 2 (11%) SNVs disrupting canonical splicing sites, 5 (26%) frameshift insertions or deletions, and 2 (11%) intragenic deletions (Table 1). One deletion encompassed exons 3 to 8 with breakpoints localized in introns 2 and 8 (patient 1) and the other one encompassed exons 2 to 7 with breakpoints localized in introns 1 and 7 (patient 16) (Fig. 1). Patient 1's deletion was detected following a gene panel analysis as previously described (Quenez et al., 2019).

Breakpoints were further characterized by analysis of misaligned reads and targeted Sanger sequencing. The deletion was confirmed using Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF ; (Saugier-Veber et al., 2006)). For patient 16, the deletion was detected following whole genome sequencing revealing multiple misaligned reads with the exact boundaries. Both deletion's breakpoints were located in Alu-type SINE repeated sequences. Both deletions were confirmed in probands and segregation analysis was performed in the parents by a targeted technique (QMPSF for patient 1 and qPCR for patient 16). All but one of the 19 pathogenic variants were *de novo* mutations, which is consistent with the sporadic presentations. Of note, the unaffected father of patient 5 was found to be a carrier of the variant in 15% of his whole blood cells DNA (Table 1).

Perinatal period

Pregnancy was uneventful for all patients except for patient 1 who presented diminished fetal movements and polyhydramnios (Table S1). He needed ventouse delivery and presented delayed resorption and clearance of fetal alveolar fluid. Patient 14 needed caesarian section delivery for dystocia. Birth weight and length were within normal ranges (between 3 and 97% or, -2 and +2 SD) for all patients with two patients showing birth weight <10% (Fig. 2A, Table S2). Birth head circumference was slightly increased with median Z-score at +1 SD (range [+0.1 - +4.7]) (Fig. 2A) and three patients presented a head circumference above 97% (P11, P13 and P19) (Table S2). Prenatal ultrasounds did not reveal any fetal anomalies.

Neurodevelopmental features

All patients presented with global psychomotor delay (Table 2, Table S1). Neonatal hypotonia was noticed in 11 patients (58%). The median ages for main developmental milestones (Fig.

2B) reflected the severity of the delay with independent sitting at 13 months (range: [9 - 21] months), independent walking at 2.5 years (range: [2 - 5] years), and first spoken words at 3 years (range: [1 - 6] years).

Speech was more severely affected with slow and poor progress (Fig. 2B, Fig. S1). Seven patients (all above 18 months of age) were not able to pronounce any words, including the older patients at the age of 35 and 30 years, (patients 15 and 2, respectively), and six patients could say words but could not associate them. Only three patients were able to make sentences albeit with limitations. Patient 17 could speak in short sentences, patient 6 could make simple sentences with abnormal speech prosody, patient 9 only started associating words at 11 years of age, he spoke in jargon and gestures at home and used pictograms at school (Table S1). Overall, comprehension seemed better than predicted by the level of verbal skills and some patients developed non-verbal communication skills (pointing, pictograms, gesture, simplified sign language) that satisfied day-to-day needs and caregiver's comprehension. These non-verbal communication skills can be surprisingly advanced as for patient 18 who is able to write short sentences but can only pronounce a few single words (Table S1).

Intellectual disability (ID) was present in all patients who could be evaluated (n = 17). ID severity was considered as moderate (n = 8), moderate to severe (n = 3), severe (n = 4) or profound (n = 1), with only one patient showing mild ID (Table 2, Fig. S1). All patients attended specialized institutions for education and often needed reeducation. Independence for older patients was partial or restricted, none of the adult patients could apply for employment even in work-based support structures.

Behavioral problems were present in 13 patients (68%), low frustration tolerance (n = 7) being the most common issue (Table 2, Table S1). Four patients presented autism spectrum disorder and another patient presented autistic features with repetitive behavior, stereotypies, restricted interest but was not ascertained by a formal evaluation by a psychiatrist (Table S1). Two patients also presented aggressive behavior, one patient had important agitation (medicated) and one patient was described as anxious. Patients were otherwise described as generally jovial or good tempered, with easy smiling and occasional inappropriate laughter for two patients (table S1).

Muscle tone and neuromotor abnormalities were also present. Seven out of fourteen patients (50%) presented hypotonia after the neonatal period, five patients (26%) had fine motor and coordination difficulties. Walking anomalies were also described, such as broad-based gait and ataxia (table S1). Patient 15 exhibited regression with transient loss of walking ability by the age of 31 years. Three patients had *pes valgus* and two patients had kyphosis in the context of hypotonia (table S1). Neurological examination revealed bilateral extensor plantar reflex in two patients, lower limb hypertonia in two patients, and transient *talipes equinovarus* in one patient. Patient 14 was treated for epilepsy and presented atonic seizures. Patient 1 presented breath-holding spells episodes that were initially incapacitating but improved with age.

Morphological features

The Z-scores distribution for height, weight and head circumference (HC) at last examination are displayed in Fig. 2A. Median weight was comparable to general population, median height was around -1 SD and HC around +2 SD. Macrocephaly, defined as HC > +2SD, was observed in 10 patients (53% ; Table 2).

Dysmorphic features were reported in 16 patients (84%). Most common features (present in more than 50% of patients) included broad forehead, deeply set eyes, hypertelorism, wide nasal base, pointed chin and blond or lighter hair (Table 2, Fig. 3). We also found patients often presented narrow palpebral fissures (47%), large/prominent noses (42%) with wide nasal bridge giving it a tubular shape for some patients (32%), periorbital fullness (41%), a broad mouth (33%) with downturned corners (39%) and thin upper lip (33%), and minor hand, feet (44%) and ear anomalies (42%). See supplementary data for additional pictures and description (table S1 and Fig. S2).

Other clinical features

Feeding difficulties were noticed in nine patients (50 %, Table 2), with one patient showing only neonatal difficulties but the other eight still presenting difficulties for chewing and swallowing during childhood and until 15 years of age for one patient (table S1). Patient 3 presented severe gastroesophageal reflux treated by Nissen surgery, esophageal achalasia, and oral disorder needing tube feeding and still required a gastrostomy at the age of 10 years.

Strabismus was present in twelve patients (63%) and other vision disorders were present in 8 out of 15 patients (53%) with mainly refractive disorders (table S1). Only one patient presented hearing impairment reported as conductive hearing loss. Organ malformations were rare and considered as minor anomalies. They included persistent arterial duct, teeth anomalies in number and shape, scoliosis, hip dislocation, and phimosis (table S1). Notably, Patient 1 presented stridor, asthma, and recurrent respiratory infections, he had one fracture of the lower extremity of the left radius following a fall. Patient 3 had an episode of transient hip synovitis, he developed acute lymphoblastic leukemia, presented hypogammaglobulinemia with recurrent infections, teeth misalignment and scoliosis. Patient 9 had supernumerary teeth,

nasal voice, one café-au-lait spot and a hypo-pigmented spot, and microcytosis on blood analysis. Patient 14 presented with several fractures with ongoing explorations. Patient 15 presented delayed puberty onset.

Brain imaging

Seventeen patients underwent brain Magnetic Resonance Imaging (MRI), five were reported as normal (Table 2). Abnormalities were reported in twelve patients (71%). We were able to study complete MRI report and/or MRI images for 14 patients. Most common abnormalities were ventriculomegaly and/or enlarged cerebrospinal fluid (CSF) spaces (43%) and white matter signal abnormalities (64%). For some patients, these white matter abnormalities could be better described as persistent hyperintense T2 in late myelination regions beyond the expected age or delayed myelination (29%) (Fig. 4; Table S1).

Discussion

Our study confirms that GAND is characterized by delayed developmental milestones, impaired speech, moderate to severe intellectual disability, macrocephaly, and white matter abnormalities on brain MRI. Speech impairment remains a predominant issue in older children and adults as reported in previous studies. In our study, 6 patients out of 17 over 4 years of age remained non-verbal (35%) which is a little more than the 7 out of 35 (22%) reported in the study from Shieh et al.(Shieh et al., 2020). Non-verbal skills should be encouraged with use of pictograms, speech generating devices and sign language whenever possible as some patients are limited by their fine motor difficulties but are inclined to communicate. Some patients can even learn to write, as Patient 18 from our study and the

patient reported by Rabin et al.(Rabin et al., 2018). Individuals with GAND seem to show good social skills and will to interact. Behavioral co-morbidities that impair communication and learning abilities such as psychomotor agitation, aggressiveness, anxiety should be managed as best and as early as possible to allow children to progress. Autism was not very common, which is consistent with previous findings (Ueda et al., 2018; Vermeulen et al., 2017) but the diagnosis is difficult to ascertain given the severe speech impairment in individuals with GAND. Individuals who present autistic features such as repetitive behavior, restricted interests or stereotyped gestures should be properly evaluated for autism spectrum disorder. In their cohort, Shieh et al. (Shieh et al., 2020) did not report on autistic features but report that most subjects had normal eye contact and social reciprocity.

Although most patients shared various clinical features, the diagnosis remains easier by a genotype-first approach. Indeed, most common clinical features are not specific enough to allow clinical recognition of GAND but are useful to validate the diagnosis after the identification of a *GATAD2B* variant. We observed that individuals with GAND shared morphological features, especially at similar ages, with broad forehead, hypertelorism or telecanthus, deeply set eyes, pointed chin and a tendency towards shortening of the philtrum, prognathism and upper lip eversion with age. Most morphological features were previously reported, especially those present in more than 40% of patients. Pointed chin was not reported in the initial study by Willemsen et al.(Willemsen et al., 2013) but was reported in most patients (91,9%) from the Shieh et al.(Shieh et al., 2020) study. When combined, the pointed chin and prominent/broad forehead can give the impression of Sotos-like facial gestalt in some patients. Other comparable features include deeply set eyes (53%) versus prominent supraorbital ridges (62.2%) and large/prominent nose (42%) versus elongated wide nose (35.1%). A short philtrum was less common in our study than in Shieh et al. (Shieh et al., 2020) (26% versus 51.3%).

Neurodevelopmental history was marked by global hypotonia that progressively improved with age, and probably participated in feeding difficulties, motor delay, oral hypotonia, and language impairment. Feeding difficulties could persist beyond neonatal period as reported before (Shieh et al., 2020; Tim-Aroon et al., 2017). Some patients presented pyramidal tract signs such as lower limb hypertonia, spasticity, Babinski sign, as reported before (Ueda et al., 2018). One patient transiently lost the ability to walk at 31 years old without any identified cause.

We were able to study brain MRI findings for most patients in this study. Observed abnormalities, mainly delayed myelination and ventriculomegaly and/or extra-ventricular enlarged CSF spaces, were unspecific but have been reported previously in individuals with *GATAD2B* pathogenic variants (Hamdan et al., 2014; Shieh et al., 2020; Ueda et al., 2018; Vanderver et al., 2016). Enlarged CSF spaces was less common than macrocephaly and was not always associated with macrocephaly.

Patient 3 presented acute lymphoblastic leukemia which was not reported before. Although the development of acute lymphoblastic leukemia in this child might be incidental, the involvement of variants affecting transcriptional regulatory genes in childhood leukemia and tumors, as well as the association of the NuRD complex with IKAROS transcription factor in early lymphopoiesis (Dege and Hagman, 2014), raises the question of a common pathogenic pathway.

Only one patient in our study had epilepsy with abnormal EEG. Epilepsy was previously reported in 14 patients (Hamdan et al., 2014; Shieh et al., 2020; Willemsen et al., 2013) and suspected in 2 (Rabin et al., 2018; Willemsen et al., 2013). If we compare to previous case reports (15.4% ; 2 out of 13), epilepsy is less frequent in our study (5.3%) and more frequent in the study from Shieh et al. (24% overall, 57.1% for missense cases, 18.6% for other variants). Epilepsy occurrence was the only significant clinical difference found in patients

with missense mutations. But given the small number of missense cases reported it is difficult to be assertive and epilepsy remains a minor feature in GAND.

Another rare feature in our study that was more frequent in the Shieh et al. study are cardiac abnormalities (10%; 5 out of 50). Given the role of the NuRD complex in heart development and particularly CHD4 (Akerberg and Pu, 2019), systematic echocardiogram could be advised.

Our study included PTC variants and intragenic deletions predicted to result in LoF. We found 53% nonsense variants which is higher than previous studies (38% in various studies and 34% in Shieh et al. study). We found similar proportions of frameshift and splice site variants. We report two intragenic deletions with characterized breakpoints in introns 1, 2, 7 and 8. The two deletions reported by Shieh et al. concerned exon 7 and exons 7 to 11, with breakpoints in intron 7 located in repeated sequences (simple $(TAGA)_n$ repeat for patient GAND50 and MIR family repeat for patient GAND16). The breakpoint from patient GAND50 is remarkably close to that of patient 16 from our study though located in a different repeated sequence. We did not recruit patients with missense variants. The first missense variants were reported by Shieh et al. (Shieh et al., 2020). They reported five *de novo* missense variants located in the CR1 and CR2 conserved domains. Three of them disrupted p66beta interactions with the NuRD complex. The development of accessible assays to test missense variants effect, associated with detailed phenotyping, will improve diagnostic yields of currently available genetic tests.

We report the fifth case of parental mosaicism for *GATAD2B* (Kaur et al., 2019; Rabin et al., 2018; Shieh et al., 2020; Willemsen et al., 2013) with potential implications in genetic counselling for parents. The recurrence risk of *de novo* mutation is usually taken into account in genetic counseling since mosaicism could be undetected in parents and affect the germinal cell line.

In conclusion, GAND is a cause of syndromic intellectual disability characterized by developmental delay, hypotonia, severe speech impairment, moderate to severe intellectual disability, common morphological features, macrocephaly, minor anomalies of the extremities, unspecific brain MRI abnormalities, and absence of major organ malformations. Some of these features, mainly developmental delay, intellectual disability, speech impairment, macrocephaly, and broad forehead, overlap with other syndromes and, interestingly, with those associated with NuRD components CHD3 (Snijders Blok et al., 2018) and CHD4 (Weiss et al., 2020, 2016). It was suggested that p66beta functions as a bridge to bind CHD proteins to the NuRD complex and target nucleosomes (Torrado et al., 2017). This could explain the clinical overlap between these syndromes and is consistent with the concept of "NuRDopathies" (Pierson et al., 2019) as epigenetic disorders sharing features with other overgrowth syndromes.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. *GATAD2B* mutations.

GATAD2B pathogenic variants from this study are depicted on schematic representation of *GATAD2B* transcript (NM _020699.2) for each patient. Variants were distributed across all

coding exons (exons 2 to 10 and beginning of exon 11). Frameshift variants are written in red, and splice variants are written in blue and preceded by a #.

Figure 2. Neurodevelopmental and growth evaluation of GAND patients.

Box plots representing age distribution in months of developmental milestones (A). Box plots of Z-scores distribution for growth measurements at birth (left) and at last examination (right). Mean (cross), median and ranges values are given beside (B). Nb: number. W: weight. L: length. HC: head circumference. H: height.

Figure 3. Facial appearance of 11 individuals with GAND.

Patients 3, 4, 6, 7, 13, 14, 15 and 18 are shown at different ages.

Figure 4. Selected brain MRI images in sagittal, axial, and coronal views.

Images from patient 1 (P1) at 4 years 5 months, patient 5 (P5) at 2 years 5 months, patient 9 (P9) at 7 years 4 months, patient 14 (P14) at 4 years 10 months, and patient 18 (P18) at 8 years. Hyperintense T2 or FLAIR white signal visible in axial and coronal views are separated from the ventricles by a myelinated zone and are more visible in anterior and posterior regions. This signal is separated from the ventricles by a hypointense stretch and partly concerns the terminal zones known for slow myelination (Barkovich, 2005). This is less visible in the older patient (P9, P18). For P1, Virchow Robin spaces appear enlarged. Presence of a discrete intrasellar meningocele in P5 (location pointed by the *).

Table 1. Summary of *GATAD2B* mutations.

GATAD2B pathogenic variants are reported for each patient, at genomic (NC_000001.11) and coding DNA (NM_020699.2) level, as well as predicted protein effect (NP_065750.1), detection method and inheritance status.

coord.: coordinates. ID: Intellectual Disability. WES: Whole Exome Sequencing. WGS: Whole Genome Sequencing.

Patient	genomic coord. [hg19]	cDNA	protein	Location	Variant detection method	Inheritance
P1	g.153784763_153796463del	c.335+4026_ 1420-155del	p.?	exons 3 to 8	ID gene panel	*de novo*
P2	g.153790571_153790572del	c.673_674del	p.(Leu225Serfs*10)	exon 5	WES	*de novo*
P3	g.153800709dup	c.115dup	p.(Ala39Glyfs*20)	exon 2	ID gene panel	*de novo*
P4	g.153785884dup	c.1261dup	p.(Ala421Glyfs*18)	exon 8	WES	*de novo*
P5	g.153785734G>A	c.1411C>T	p.(Gln471*)	exon 8	ID gene panel	paternal mosaicism (15%)
P6	g.153784318G>A	c.1537C>T	p.(Gln513*)	exon 10	WES	*de novo*
P7	g.153791266C>T	c.597+1G>A	p.?	intron 4	WES	*de novo*
P8	g.153788766_153788767del	c.1198_1199del	p.(Ser400Cysfs*3)	exon 7	ID gene panel	*de novo*
P9	g.153784596G>A	c.1432C>T	p.(Arg468*)	exon 9	ID gene panel	*de novo*
P10	g.153791329G>A	c.535C>T	p.(Arg179*)	exon 4	ID gene panel	*de novo*
P11	g.153788992G>A	c.973C>T	p.(Gln325*)	exon 7	WES	*de novo*
P12	g.153784599G>A	c.1429C>T	p.(Gln477*)	exon 9	WES	*de novo*
P13	g.153800553dupT	c.271dupA	p.(Thr91Asnfs*12)	exon 2	WES	*de novo*
P14	g.153785728G>A	c.1417C>T	p.(Gln473*)	exon 8	clinical WES/ID panel	*de novo*
P15	g.153785929C>G	c.1217-1G>C	p.?	intron 7	ID panel	*de novo*
P16	g.153788317_153802363del	c.-1-1539_ 1216+432del	p.?	exons 2 to 7	WGS	*de novo*
P17	g.153784590G>A	c.1438C>T	p.(Gln480*)	exon 9	WES	*de novo*
P18	g.153789907T>A	c.841A>T	p.(Lys281*)	exon 6	ID panel	*de novo*
P19	g.153785899C>A	c.1246G>T	p.(Glu416*)	exon 8	WES	*de novo*

Table 2. Summary of clinical findings.

	Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	Count/Med
	Gender	M	M	M	F	M	M	F	M	M	M	M	F	M	M	F	F	F	F	M	M/F : 12/7
	Age at diagnosis	5	30	8	5	1.6	6.7	2.8	4.5	13.42	8	7	7.5	14.5	4.2	36	4	12	14	12	Med = 7.5y
Perinatal	**Postmenstrual age (wks)**	39	40	38	38	39	39	40	37 + 6	38	41	38	38	38	39	at term	41	42	41 + 2	38	
	Apgar score at 3/5 min	2/6	10/10	10/10	10/10	10/10	10/10	NA	10/10	10/10	10/10	10/10	10/10	NK	NK	10/10	4/9/10	7/9/10	9/10	NK	
	Birth weight (g)	2830	3100	3580	2980	3400	2980	3431	2800	2610	4100	3140	2840	3800	3350	3800	3400	4075	4000	3650	Med = 3400 g
	Birth length (cm)	50	50	50	48.5	50	51.5	51	49.5	46	52	52	51	50	50	50	51	55	54.5	52	Med = 50 cm
	Birth HC (cm)	35.5	37.5	36	35	NK	35.8	NK	36	34.5	36	37.5	35	41	NK	NK	36	38	37	41	Med = 36 cm
Neurodevelopement	**Neonatal hypotonia**	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	11/19 (58%)
	Motor delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19/19 (100%)
	Sitting age	10 m	NA	9 m	13 m	13 m	9 m	14 m	>1y	>1y	18m	18m	18m	12m	11m	NK	21m	14m	14m	12m	Med = 13m
	Walking age	24 m	24 m	26 m	29 m	NA	27 m	3y 6m	2y 6m	5y	3y	5y	2y 9m	3y	2y 6m	3y	4y	24m	22m	2y 6m	Med = 2.5y
	Speech delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19/19 (100%)
	Age at first words	2.5y	NA	3y	18 m	NA	<1y	3.5 - 4y	NA	3y	4y	6y	NA	NA	NA	4y 10m	NA	18m	18m	NK	Med = 3y
	Intellectual Deficiency	+	+	+	+	NA	+	+	NK	+	+	+	+	+	+	+	+	+	+	+	17/17 (100%)
	Severity	++	++++	++	++	NA	+	++	NK	+++	+++	++	++	+++++	+++	++++	++++	++	++	++++	
	Behavioral problems	+	-	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	+	+	13/19 (68%)
Oher	**Feedind difficulties**	-	-	+	-	+	+	+	NK	+	-	-	+	+	+	-	+	-	-	-	9/18 (50%)
	Sleep disorder	-	-	NK	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	3/18 (17%)
	Constipation	+	-	Nk	+	-	-	+	NK	-	-	-	-	+	-	-	-	-	-	NK	4/16 (25%)
Senses	**Deafness**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1/19 (5%)
	Vision disorder	+	-	-	-	NK	+	+	NK	+	-	+	+	-	-	-	NK	+	+	+	9/16 (63%)
	Strabismus	+	-	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	12/19 (63%)
Brain MRI	**Abnormalities**	+	-	+	NK	+	+	+	-	-	+	+	-	+	+	NK	+	-	+	+	12/17 (71%)
	white matter signal	+	-	+	NA	-	-	-	NA	+	+	+	NA	-	+	NA	+	NA	+	+	9/14 (64%)
	myelination delay	-	-	-	NA	-	-	-	NA	-	+	-	NA	-	+	NA	+	NA	+	-	4/14 (29%)
	ventriculomegaly ± enlarged CSF space	-	-	+	NA	-	+	+	NA	-	-	+	NA	+	-	NA	-	NA	+	-	6/14 (43%)
	other	-	-	+	NA	+	+	-	NA	-	-	-	NA	+	-	NA	-	NA	-	+	5/14 (36%)

Table 2. Summary of clinical findings (continued).

Presence or absence of main features for each patient. Counts and corresponding percentages are reported for qualitative findings and the median for quantitative data. SD: standard deviation. HC: Head Circumference. +: present. -: absent. Med: median. M: male. F: Female. Wks: weeks. m: months. y: years. g: grams. cm: centimeters. NA: not applicable or not acquired. NK: not known. Intellectual disability's severity: + is mild, ++ is moderate, +++ is moderate to severe, and ++++ is severe, and +++++ is profound.

	Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	Count/Med
Growth	**Age at last examination**	4y	28y	10y	4y 5m	2y10 m	7y7m	4y	3y 9m	13y 5m	8y 10m	6y	7y 6m	15y 6m	5y 6m	35y	4y 4m	NK	13	11	Med = 7.5y
	Weight (SD)	+2	+1	-1	0	+0.5	0	-2	0	-2.5	-0.8	+0.5	-0.4	-2.3	+0.58	NK	-1.75	0	+1.5	+0.5	Med = 0 SD
	Height (SD)	+1.5	-1	-1	-1	+1	+1	-2.5	-1.5	-2	-1.5	+0.75	-1	-2.5	-0.64	NK	-0.68	0	+2	+0	Med = -0.8 SD
	HC (SD)	+2.5	+3.5	+3	+2	+2	+1.5	+1.5	+3	-1.5	+1.5	+4	-0.38	+3.5	+1.72	+2.34	+2.45	0	+3.4	+2.5	Med = +2.3 SD
	Macrocephaly	+	+	+	-	-	-	-	+	-	-	+	-	+	+/-	+	+	-	+	+	10/19 (53%)
	Dysmorphic features	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	16/19 (84%)
Upper face	Broad forehead	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	16/19 (84%)
	Narrow palpebral fissures	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+	+	-	-	-	9/18 (50%)
	Deeply set eyes	+	+	+	+	-	+	+	-	+	-	-	-	-	-	+	+	-	+	-	10/19 (53%)
	Hypertelorism	+	-	+	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	14/19 (74%)
	Periorbital fullness	+	-	-	-	NK	-	-	+	+	-	-	+	+	-	+	-	-	+	NK	7/17 (41%)
Middle face	Ear anomalies	-	+	-	+	-	+	+	-	+	-	-	+	-	+	-	-	-	+	-	8/19 (42%)
	Large/prominent nose	+	+	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	8/19 (42%)
	Tubular shaped nose	+	-	+	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	6/19 (32%)
	Wide nasal base	-	-	+	+	-	-	+	+	+	-	+	-	+	+	NK	+	-	+	+	11/18 (61%)
Lower face	Short Philtrum	-	-	-	-	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	5/19 (26%)
	Broad mouth	-	-	-	-	-	-	+	+	-	-	-	-	+	+	NK	-	-	+	+	6/18 (33%)
	Downturned mouth	-	+	+	-	-	+	+	+	+	-	-	-	-	+	NK	-	-	-	-	7/18 (39%)
	Thin upper lip	-	-	+	-	-	-	-	+	+	-	-	-	+	-	NK	+	-	+	-	6/18 (33%)
	pointed chin	+	NK	+	+	+	+	+	NK	+	NK	NK	NK	+	+	+	-	-	+	-	11/14 (79%)
	Blond hair	+	-	+	-	-	-	-	+	+	+	-	+	+	+	-	+	-	-	-	9/18 (50%)
	Thin hair	-	+	-	-	+	-	NK	-	-	-	-	+	+	-	NK	+	-	-	-	5/17 (29%)
	Hands/ feet anomalies	+	-	+	+	+	+	-	-	+	-	-	+	-	-	-	-	NK	+	-	8/18 (44%)
	Long fingers	-	-	-	-	-	NK	-	-	+	-	-	-	-	-	-	-	NK	+	+	3/16 (19%)
	Clinodactyly	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	NK	-	-	2/17 (12%)

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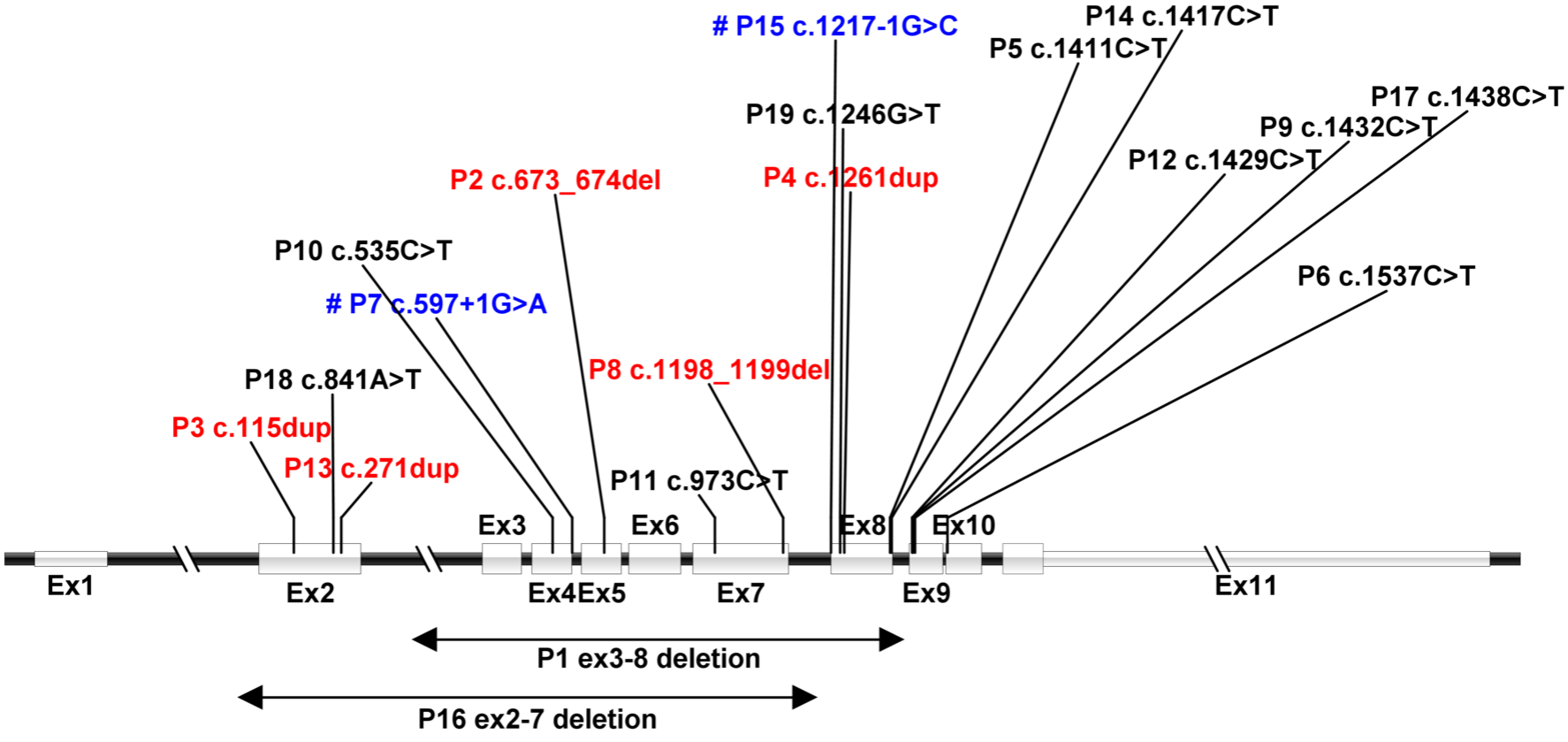
Supplementary Data

Table S1. Detailed clinical findings for all patients.

Table S2. Birth measurements, calculated Z-scores, and percentiles for given postmenstrual age.

Figure S1. Language and intellectual disability at last evaluation.

Figure S2. Additional hands and feet pictures from patients.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P7 c.597+1G>A
P8 c.1198_1199del
P18 c.841A>T
P3 c.115dup
P13 c.271dup
P11 c.973C>T
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Ex10
Ex1
Ex2
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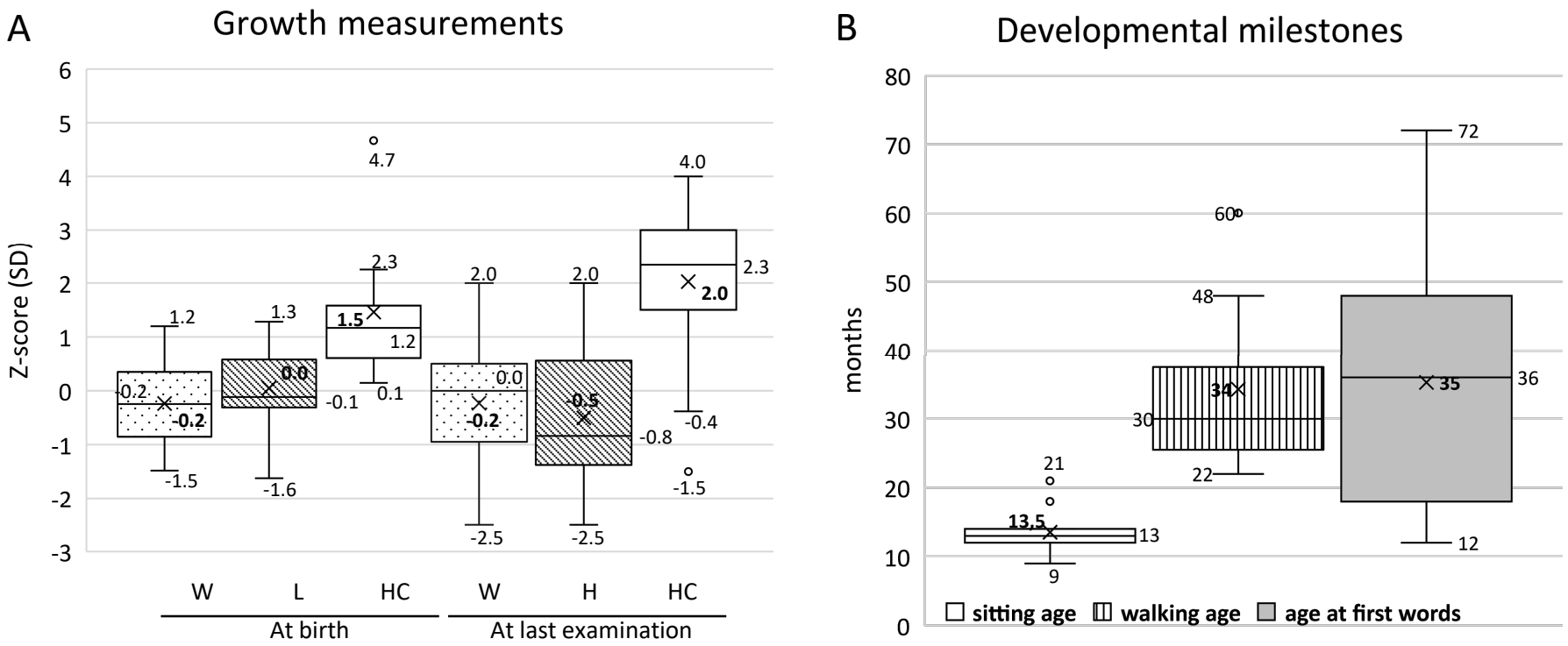
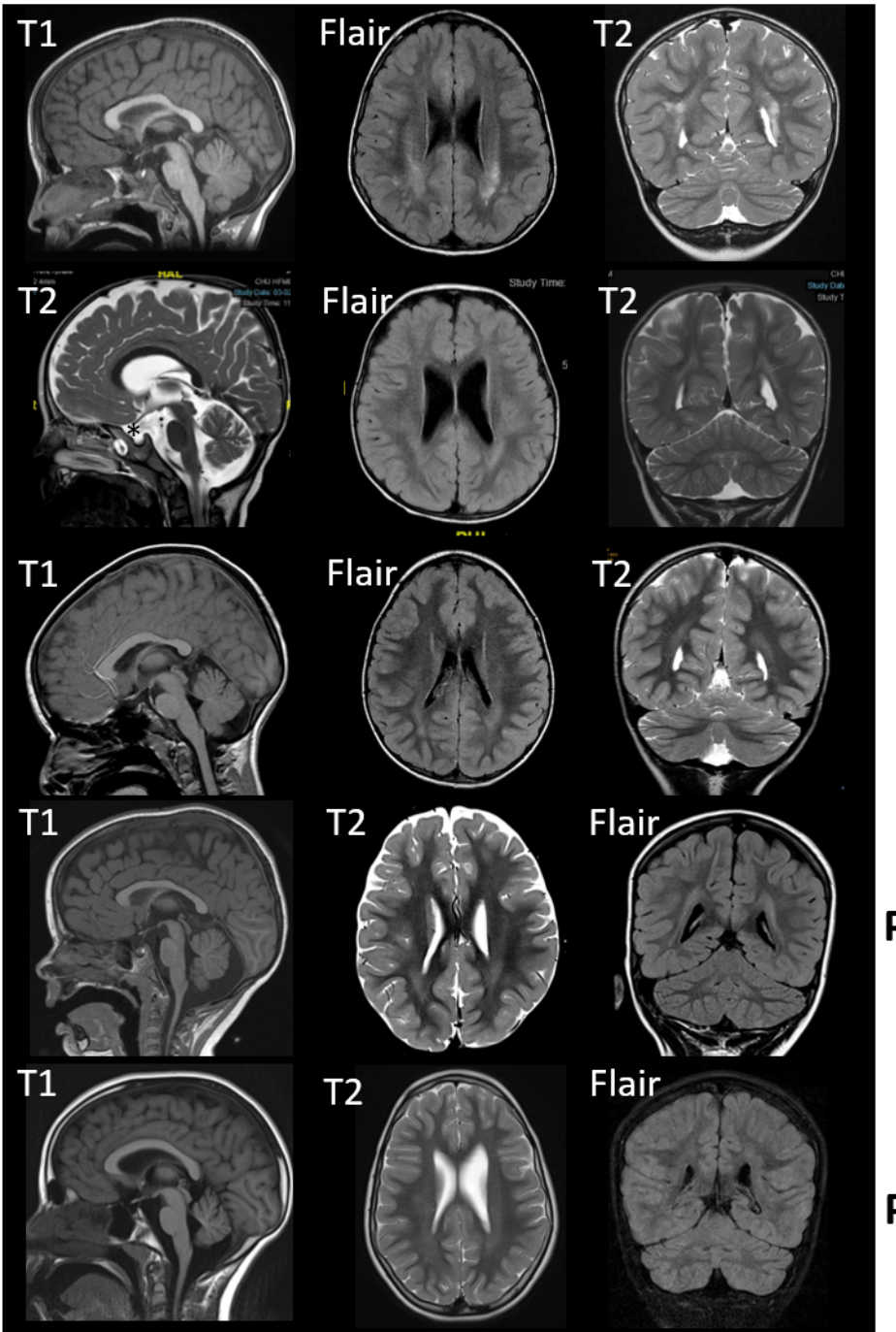

A
Growth measurements
Z-score (SD)
6
5
4
3
2
1
0
-1
-2
-3
1.2
0.2
-0.2
-1.5
1.3
0.0
-0.1
-1.6
4.7
2.3
1.5
1.2
0.1
2.0
0.0
-0.2
-2.5
2.0
0.5
-0.8
-2.5
4.0
2.3
2.0
-0.4
-1.5
W
L
HC
W
H
HC
At birth
At last examination
B
Developmental milestones
months
80
70
60
50
40
30
20
10
0
72
60
48
36
35
34
30
22
21
13.5
13
9
12
sitting age
walking age
age at first words


P1
P3
P13
P4
P5
P14
P6
P15
P7
P18
P9


T1
Flair
T2
P1
T2
Flair
T2
P5
T1
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P9
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T1
T2
Flair
P18